

\$V

DOCKET NO.: CELL-0271

PATENT



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Julien Alistair Brown, Stuart Bailey and
Stephen Brand

Confirmation No.:

Application No.: 10/620,533

Group Art Unit:

Filed: July 16, 2003

Examiner:

For: Phenylalanine Enamide Derivatives

DATE OF DEPOSIT: *August 28, 2003*

I HEREBY CERTIFY THAT THIS PAPER IS BEING
DEPOSITED WITH THE UNITED STATES POSTAL
SERVICE AS FIRST CLASS MAIL, POSTAGE PREPAID,
ON THE DATE INDICATED ABOVE AND IS
ADDRESSED TO THE COMMISSIONER FOR PATENTS,
P.O. BOX 1450, ALEXANDRIA, VA 22313-1450.

Jane E. Inglesse

TYPED NAME: Jane E. Inglesse, Ph.D.
REGISTRATION NO.: 48,444

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

**TRANSMITTAL OF CERTIFIED COPY OF PRIORITY APPLICATION
PURSUANT TO 37 CFR § 1.55**

Attached please find the certified copy/copies of the foreign application from which
priority is claimed for this case:

Country:	Application No.:	Filing Date:
United Kingdom	GB 0216568.6	July 17, 2002



The fee of \$130.00 for entry of late priority documents is enclosed herewith. Please
charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

00000000 10620533

SEP 16 2003

10/620,533

DOCKET NO.: CELL-0271

PATENT

☒ The Commissioner is hereby authorized to charge payment of the above fees associated with this communication or credit any overpayment to Deposit Account No. 23-3050. This sheet is attached in duplicate.

Date: *August 28, 2003*

Jane E. Inglese
Jane E. Inglese, Ph.D.
Registration No. 48,444

Woodcock Washburn LLP
One Liberty Place - 46th Floor
Philadelphia PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

© 2003 WW



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or the equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Andrew Jones

Dated 7 July 2003

THE PATENT OFFICE
A
17 JUL 2002



1/77

17JUL02 E733869-2 C72481
P01/7700 0.00-0216568.6

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference

PA 5148

2. Pa

(n

0216568.6

17 JUL 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

CELLTECH R&D LIMITED,
208, BATH ROAD,
SLOUGH,
SL1 3WE

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

UK

8121485001

4. Title of the invention

CHEMICAL COMPOUNDS

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

~~FROTH-KENALL,~~
~~CELLTECH R&D LTD,~~
~~ABINGDON,~~
~~CAMBRIDGE,~~
~~CB1 6ES~~

CALPANELS & LANSFORD,
43, BUCKINGHAM SQUARE,
LONDON
WC1A 2RA

AFS:J77
10/6/03
JHOCME

Patents ADP number (if you know it)

~~8164428002~~ 83001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

4E9

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form ☐

Description 16 /

Claim(s) ☐

Abstract ☐

Drawing(s) ☐

10. If you are also filing any of the following, state how many against each item.

Priority documents ☐

Translations of priority documents ☐

Statement of inventorship and right to grant of a patent (Patents Form 7/77) ☐

Request for preliminary examination and search (Patents Form 9/77) ☐

Request for substantive examination (Patents Form 10/77) ☐

Any other documents ☐
(please specify)

11. I/We request the grant of a patent on the basis of this application.

FOR AND ON BEHALF OF CELTECH R & D LTD
Signature Date

M. J. Kendall

16th JULY 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

H. KENDALL

01223 896499

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

CHEMICAL COMPOUNDS

This invention relates to a number of phenylalanine enamide esters, to compositions containing them, to processes for their preparation, and to
5 their use in medicine.

Over the last few years it has become increasingly clear that the physical interaction of inflammatory leukocytes with each other and other cells of the body plays an important role in regulating immune and inflammatory
10 responses [Springer, T. A., *Nature*, 346, 425, (1990); Springer, T. A., *Cell*, 76, 301, (1994)]. Specific cell surface molecules collectively referred to as cell adhesion molecules mediate many of these interactions.

The adhesion molecules have been sub-divided into different groups on
15 the basis of their structure. One family of adhesion molecules which is believed to play a particularly important role in regulating immune and inflammatory responses is the integrin family. This family of cell surface glycoproteins has a typical non-covalently linked heterodimer structure. At least 16 different integrin alpha chains and 8 different integrin beta chains
20 have been identified [Newman, P. *et al*, *Molecular Medicine Today*, 304, (1996)]. The members of the family are typically named according to their heterodimer composition although trivial nomenclature is widespread in the field. Thus the integrin $\alpha 4\beta 1$ consists of the integrin alpha 4 chain associated with the integrin beta 1 chain, but is also widely referred to as
25 Very Late Antigen 4 or VLA-4. Not all of the potential pairings of integrin alpha and beta chains have yet been observed in nature and the integrin family has been subdivided into a number of subgroups based on the pairings that have been recognised to date [Sonnenberg, A., *Current Topics in Microbiology and Immunology*, 184, 7, (1993)].

30 The importance of integrin function in normal physiological responses is highlighted by two human deficiency diseases in which integrin function is defective. Thus in the disease termed Leukocyte Adhesion Deficiency (LAD) there is a defect in one of the families of integrins expressed on
35 leukocytes [Marlin, S. D. *et al*, *J. Exp. Med.* 164, 855, (1986)]. Patients suffering from this disease have a reduced ability to recruit leukocytes to

inflammatory sites and suffer recurrent infections, which in extreme cases may be fatal. In the case of patients suffering from the disease termed Glanzman's thrombasthenia (a defect in a member of the beta 3 integrin family) there is a defect in blood clotting (Hodivala-Dilke, K. M., J. Clin. Invest. 103, 229, (1999)).

The potential to modify integrin function in such a way as to beneficially modulate cell adhesion has been extensively investigated in animal models using specific antibodies and peptides that block various functions of these molecules [e.g. Issekutz, T. B., J. Immunol. 149, 3394, (1992); Li, Z. *et al*, Am. J. Physiol. 263, L723, (1992); Mitjans, F. *et al*, J. Cell Sci. 108, 2825, (1995); Brooks, P. C. *et al*, J. Clin. Invest. 96, 1815, (1995); Binns, R. M. *et al*, J. Immunol. 157, 4094, (1996); Hammes, H.-P. *et al*, Nature Medicine 2, 529, (1996); Srivata, S. *et al*, Cardiovascular Res. 36, 408 (1997)]. In particular an anti $\alpha_4\beta_7$ -antibody has demonstrated both clinical and histologic improvement of inflammatory activity and disease in a non-human primate model of inflammatory bowel disease [Hesterberg, P.E. *et al*, Gastroenterol, 111, 1373-80 (1996)]. A number of monoclonal antibodies which block integrin function are currently being investigated for their therapeutic potential in human disease, and one, ReoPro, a chimeric antibody against the platelet integrin $\alpha_{IIb}\beta_3$ is in use as a potent anti-thrombotic agent for use in patients with cardiovascular complications following coronary angioplasty.

Integrins recognize both cell surface and extracellular matrix ligands, and ligand specificity is determined by the particular alpha-beta subunit combination of the molecule [Newman, P., *ibid*]. One particular integrin subgroup of interest involves the α_4 chain which can pair with two different beta chains β_1 and β_7 [Sonnenberg, A., *ibid*]. The $\alpha_4\beta_1$ pairing occurs on many circulating leukocytes (for example lymphocytes, monocytes, eosinophils and basophils) although it is absent or only present at low levels on circulating neutrophils. $\alpha_4\beta_1$ binds to an adhesion molecule (Vascular Cell Adhesion Molecule-1 also known as VCAM-1) frequently up-regulated on endothelial cells at sites of inflammation [Osborne, L., Cell, 62, 3, (1990)]. The molecule has also been shown to bind to at least three sites in the matrix molecule fibronectin [Humphries,

M. J. *et al*, Ciba Foundation Symposium, 189, 177, (1995)]. Based on data obtained with monoclonal antibodies in animal models it is believed that the interaction between $\alpha 4\beta 1$ and ligands on other cells and the extracellular matrix plays an important role in leukocyte migration and activation [Yednock, T. A. *et al*, Nature, 356, 63, (1992); Podolsky, D. K. *et al*, J. Clin. Invest. 92, 372, (1993); Abraham, W. M. *et al*, J. Clin. Invest. 93, 776, (1994)].

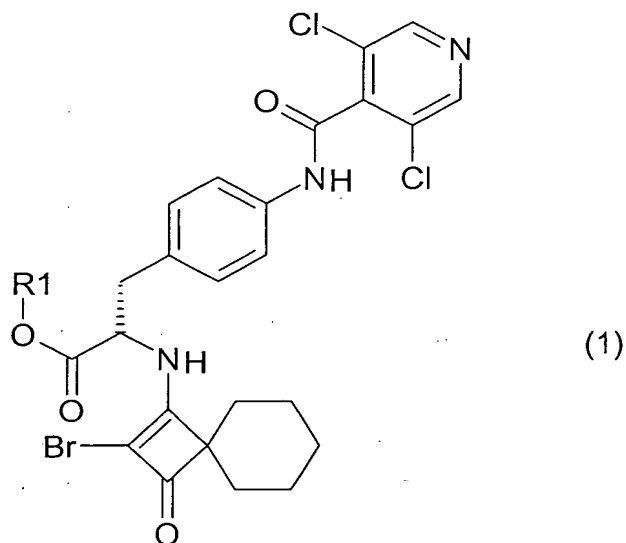
The integrin generated by the pairing of $\alpha 4$ and $\beta 7$ has been termed LPAM-1 [Holzmann, B. and Weissman, I. L., EMBO J. 8, 1735, (1989)]. The $\alpha 4\beta 7$ pairing is expressed on certain sub-populations of T and B lymphocytes and on eosinophils [Erle, D. J. *et al*, J. Immunol. 153, 517 (1994)]. Like $\alpha 4\beta 1$, $\alpha 4\beta 7$ binds to VCAM-1 and fibronectin. In addition, $\alpha 4\beta 7$ binds to an adhesion molecule believed to be involved in the homing of leukocytes to mucosal tissue such as gastrointestinal mucosa termed MAdCAM-1 [Berlin, C. *et al*, Cell, 74, 185, (1993)]. MAdCAM-1 is preferentially expressed in the gastrointestinal track. The interaction between $\alpha 4\beta 7$ and MAdCAM-1 may also be important at sites of inflammation outside of mucosal tissue [Yang, X.-D. *et al*, PNAS, 91, 12604, (1994)].

Regions of the peptide sequence recognized by $\alpha 4\beta 1$ and $\alpha 4\beta 7$ when they bind to their ligands have been identified. $\alpha 4\beta 1$ seems to recognise LDV, IDA or REDV peptide sequences in fibronectin and a QIDSP sequence in VCAM-1 [Humphries, M. J. *et al*, *ibid*] whilst $\alpha 4\beta 7$ recognises a LDT sequence in MAdCAM-1 [Birskin, M. J. *et al*, J. Immunol. 156, 719, (1996)]. There have been several reports of inhibitors of these interactions being designed from modifications of these short peptide sequences [Cardarelli, P. M. *et al*, J. Biol. Chem., 269, 18668, (1994); Shorff, H. N. *et al*, Biorganic Med. Chem. Lett., 6, 2495, (1996); Vanderslice, P. *et al*, J. Immunol., 158, 1710, (1997)]. It has also been reported that a short peptide sequence derived from the $\alpha 4\beta 1$ binding site in fibronectin can inhibit a contact hypersensitivity reaction in a trinitrochlorobenzene sensitised mouse [Ferguson, T. A., *et al*, PNAS, 88, 8072, (1991)].

Since the alpha 4 subgroup of integrins are predominantly expressed on leukocytes their inhibition can be expected to be beneficial in a number of immune or inflammatory disease states. However, because of the ubiquitous distribution and wide range of functions performed by other members of the integrin family it is important to be able to identify selective inhibitors of the alpha 4 subgroup.

We have now found a number of esters which are potent and selective inhibitors of $\alpha 4$ integrins. The compounds are able to inhibit $\alpha 4$ integrins such as $\alpha 4\beta 1$ and/or $\alpha 4\beta 7$, in for example cellular assays such as those described herein, at concentrations at which they generally have no or minimal inhibitory action on α integrins of other subgroups.

Thus according to one aspect of the invention we provide a compound of formula (1):



wherein R^1 is a $-\text{CH}_3$, $-(\text{CH}_2)_3\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{OCH}_3$, $-\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$,

$-\text{CH}_2\text{CH}_2-\text{N} \begin{array}{c} \diagup \diagdown \\ \text{O} \end{array} \diagup \diagdown$ or $-\text{CH}_2\text{CH}_2-\text{N} \begin{array}{c} \diagup \diagdown \\ \text{N}-\text{CH}_3 \end{array} \diagup \diagdown$

group;

and the salts, solvates and N-oxides thereof.

It will be appreciated that compounds of formula (1) may exist as enantiomers or diastereomers. The invention is to be understood to
 5 extend to all such enantiomers, diastereomers and mixtures thereof, including racemates. Formula (1) is intended to represent all individual isomers and mixtures thereof, unless stated or shown otherwise.

Salts of compounds of the invention include pharmaceutically acceptable
 10 salts, for example acid addition salts derived from inorganic or organic acids.

Acid addition salts include hydrochlorides, hydrobromides, hydroiodides, alkylsulphonates, e.g. methanesulphonates, ethanesulphonates, or
 15 isothionates, arylsulphonates, e.g. p-toluenesulphonates, besylates or napsylates, phosphates, sulphates, hydrogen sulphates, acetates, trifluoroacetates, propionates, citrates, maleates, fumarates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

20 Particularly useful salts of compounds according to the invention include pharmaceutically acceptable salts, especially acid addition pharmaceutically acceptable salts.

A compound of the invention which is:

25 2-Methoxyethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof;

2-(2-Hydroxyethoxy)ethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the
 30 salts, solvates and N-oxide thereof;

2-(2-Methoxyethoxy)ethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof;

2-(Morpholin-4-yl)ethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the
 35 salts, solvates and N-oxide thereof;

2-(4-Methylpiperazin-1-yl)ethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof.

- 5 A more particular compound of the invention which is:
 Butyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof;
 2-Hydroxyethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-
 10 [(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof;
 Methyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate and the salts, solvates and N-oxide thereof.

15 The compounds according to the invention are potent and selective inhibitors of α_4 integrins. The ability of the compounds to act in this way may be simply determined by employing tests such as the cellular assays described in the Examples hereinafter.

20 The compounds are of use in modulating cell adhesion and in particular are of use in the prophylaxis and treatment of diseases or disorders including inflammation in which the extravasation of leukocytes plays a role and the invention extends to such a use and to the use of the
 25 compounds for the manufacture of a medicament for treating such diseases or disorders,

Diseases or disorders of this type include inflammatory arthritis such as rheumatoid arthritis, vasculitis or polydermatomyositis, multiple sclerosis,
 30 allograft rejection, diabetes, inflammatory dermatoses such as psoriasis or dermatitis, asthma and inflammatory bowel disease.

For the prophylaxis or treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions, and
 35 according to a further aspect of the invention we provide a pharmaceutical

composition which comprises a compound of formula (1) together with one or more pharmaceutically acceptable carriers, excipients or diluents.

- 5 Pharmaceutical compositions according to the invention may take a form suitable for oral, buccal, parenteral, nasal, topical or rectal administration, or a form suitable for administration by inhalation or insufflation.

10 For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets
15 may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations
20 may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

- 25 Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

30

The compounds for formula (1) may be formulated for parenteral administration by injection e.g. by bolus injection or infusion. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoule or multi dose containers, e.g. glass vials. The compositions for injection
35 may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as

suspending, stabilising, preserving and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

- 5 In addition to the formulations described above, the compounds of formula (1) may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.
- 10 For nasal administration or administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of suitable propellant, e.g. dichlorodifluoromethane, trichloro-fluoromethane, dichlorotetrafluoroethane, carbon dioxide or other
- 15 suitable gas or mixture of gases.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by

20 instructions for administration.

The quantity of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, and the condition of the patient to be treated. In general,

25 however, daily dosages may range from around 100ng/kg to 100mg/kg e.g. around 0.01mg/kg to 40mg/kg body weight for oral or buccal administration, from around 10ng/kg to 50mg/kg body weight for parenteral administration and around 0.05mg to around 1000mg e.g. around 0.5mg to around 1000mg for nasal administration or administration

30 by inhalation or insufflation.

The esters of formula (1) may be prepared by the processes described in the Examples hereinafter.

- 35 In addition, N-oxides of compounds of formula (1) may be prepared for example by oxidation of the corresponding nitrogen base using an

oxidising agent such as hydrogen peroxide in the presence of an acid such as acetic acid, at an elevated temperature, for example around 70°C to 80°C, or alternatively by reaction with a peracid such as peracetic acid in a solvent, e.g. dichloromethane, at ambient temperature.

5

Salts of compounds of formula (1) may be prepared by reaction of a compound of formula (1) with an appropriate acid in a suitable solvent or mixture of solvents e.g. an organic solvent such as an ether e.g. diethylether, or an alcohol, e.g. ethanol using conventional procedures.

10

Where it is desired to obtain a particular enantiomer of a compound of formula (1) this may be produced from a corresponding mixture of enantiomers using any suitable conventional procedure for resolving enantiomers.

15

Thus for example diastereomeric derivatives, e.g. salts, may be produced by reaction of a mixture of enantiomers of formula (1) e.g. a racemate, and an appropriate chiral compound, e.g. a chiral base. The diastereomers may then be separated by any convenient means, for example by crystallisation and the desired enantiomer recovered, e.g. by treatment with an acid in the instance where the diastereomer is a salt.

20

In another resolution process a racemate of formula (1) may be separated using chiral High Performance Liquid Chromatography. Alternatively, if desired a particular enantiomer may be obtained by using an appropriate chiral intermediate in one of the processes described above. Alternatively, a particular enantiomer may be obtained by performing an enantiomer specific enzymatic biotransformation e.g. an ester hydrolysis using an esterase and then purifying only the enantiomerically pure hydrolysed acid from the unreacted ester antipode.

25

30

Chromatography, recrystallisation and other conventional separation procedures may also be used with intermediates or final products where it is desired to obtain a particular geometric isomer of the invention.

35

The following Examples illustrate the preparation of compounds of the invention. All temperatures are in °C. The following abbreviations are used:

- | | | |
|----|---|-------------------------------|
| | NMM - N-methylmorpholine; | EtOAc - ethyl acetate; |
| 5 | MeOH - methanol; | BOC - butoxycarbonyl; |
| | DCM - dichloromethane; | AcOH - acetic acid; |
| | DIPEA - diisopropylethylamine; | EtOH - ethanol; |
| | Pyr - pyridine; | Ar - aryl; |
| | DMSO - dimethylsulphoxide; | iPr - isopropyl; |
| 10 | Et ₂ O - diethylether; | Me - methyl; |
| | THF - tetrahydrofuran, | DMF - N,N-dimethylformamide; |
| | Fmoc - 9-fluorenylmethoxycarbonyl; | |
| | DBU - 1,8-Diazabicyclo[5,4,0]undec-7-ene; | |
| | DMAP - 4-(dimethylamino)pyridine. | HOBT - 1-hydroxybenzotriazole |

15

All NMR's were obtained either at 300MHz or 400MHz.

- All Intermediates and Examples were named with the aid of Beilstein Autonom (available from MDL Information Systems GmbH, Therdor-Heuss-Allee 108D 60486, Frankfurt, Germany) or were given names that
- 20 seemed consistent, with the exception that propanoates were named by the IUPAC name rather than the trivial name (propionate) and isonicotinoyl (trivial name) is used in place of pyridine-4-carbonyl.

INTERMEDIATE 1

25 3,5-Dichloropyridine-4-carboxylic acid

- A solution of 3,5-dichloropyridine (5.00g, 33.8mmol) in THF (25ml) was added to a solution of LDA [generated from nBuLi (2.5M solution in hexanes, 14.9ml, 37.2mmol) and diisopropylamine (4.10g, 5.7ml, 40.6mmol)] in THF (25ml) at -78° under nitrogen, to give a yellow/brown
- 30 slurry. The reaction was stirred for 30min at -78° then CO₂ gas was bubbled through to give a clear brown solution that slowly gave a precipitate, warmed to room temperature over 2h, then quenched with water (20ml) and partitioned between Et₂O (100ml) and 1M NaOH (100ml). The aqueous layer was separated and acidified to pH 1 with
- 35 concentrated hydrochloric acid and then extracted with 10% MeOH in

DCM (100mlx3). The combined organic layers were dried (MgSO₄) and the solvent removed under vacuum to give a brown solid that was recrystallised from ethanol and dried under vacuum to give the title compound as pinkish crystals (2.63g, 41%). δ H (DMSO-d₆) 8.74 (2H, s).

5 δ C (DMSO-d₆) 163.5, 147.7, 141.0, 126.7.

INTERMEDIATE 2

Ethyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

10 A slurry of the compound of Intermediate 1 (51.2g, 0.267mol) in DCM (195ml) and thionyl chloride (195ml, 2.67mol) was treated with DMF (5 drops) and heated to reflux for 4h. The reaction was concentrated *in vacuo* and azeotroped with toluene (2x50ml) to give a yellow solid which was used without further purification. A solution of ethyl-(S)-3-(4-aminophenyl)-2-(t-butoxycarbonylamino)propanoate (130.8g, 0.425mol) in
15 DCM (800ml) was cooled to 0° and treated with NMM (56.0ml, 0.51mol), stirred for 5 minutes and then a solution of the acid chloride (98.3g, 0.468mol) in DCM (200ml) was added dropwise keeping the reaction temperature below 5°. The reaction was stirred for 1h, quenched with
20 NaHCO₃ solution (500ml), the organic layer separated, washed with NaHCO₃ solution (500ml), 10% citric acid solution (500ml) and NaHCO₃ solution (500ml), dried (MgSO₄) and concentrated *in vacuo* to give a yellow solid which was recrystallised (EtOAc/hexane) to give the title compound, (140g, 69%). δ H (DMSO d₆), 8.8 (2H, s), 7.55 (2H, d, \downarrow 8.5Hz), 7.23 (2H, d, \downarrow 8.5Hz), 4.0 (3H, m), 3.4 (2H, b s), 2.9 (1H, m), 2.8 (1H, m), 1.3 (9H, s), 1.25 (3H, t); m/z (ES⁺, 70V) 504 (MNa⁺).

INTERMEDIATE 3

Ethyl (2S)-2-amino-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate hydrochloride

30 A solution of the compound of Intermediate 2 (70g, 0.146mol) in EtOAc (500ml) and 1,4-dioxan (50ml) was treated with a solution of HCl in EtOAc (500ml, 3M), and stirred at room temperature for 4h. The reaction was concentrated *in vacuo* to give a yellow solid which was triturated with Et₂O

then recrystallised (EtOAc/hexane) to give the title compound (59.3g, 92%). δ H (DMSO d_6), 11.10 (1H, s), 8.70 (2H, s), 7.55 (2H, d, J 8.4Hz), 7.25 (2H, d, J 8.4Hz), 4.10 (3H, m), 3.10 (2H, m), 1.10 (3H, m); m/z (ES⁺, 70V) 382 (MH⁺).

5

INTERMEDIATE 4

Ethyl (2S)-2-(3-oxospiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

A solution of 1-keto-3-hydroxyspiro[3.5]non-2-ene (400mg, 2.6mmol) [prepared according to the method of Wasserman, H.H. *et al*, J. Org. Chem., 38, 1451-1455 (1973)] and the free amine of intermediate 3 (400mg, 1.04mmol) in THF (10ml) was stirred at room temperature for 48h. The volatiles were removed *in vacuo* and the residue chromatographed (SiO₂; EtOAc) to give the title compound as a white powder (512mg, 0.99mmol, 95%). δ H (CDCl₃, 300K) 10.86 (1H, s), 8.78 (2H, s), 8.34 (1H, d, J 8.5Hz), 7.56 (2H, d, J 8.5Hz), 7.25 (2H, d, J 8.5Hz), 4.36 (1H, s), 4.20-4.11 (3H, m), 3.13 (1H, dd, J , 13.8, 5.3Hz), 3.00 (1H, dd, J , 9.2, 13.8Hz), 1.67-1.19 (10H, m), 1.17 (3H, t, J , 4.1Hz); m/z (ES⁺, 70V) 516.0 and 518.0 (MH⁺).

20

INTERMEDIATE 5

Ethyl (2S)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

A solution containing Intermediate 4 (500mg, 0.97mmol) and triethylamine (2eq, 270 μ l) in THF (10ml) at 0° was treated dropwise with a solution of bromine (1.1eq, 170mg) in THF (5ml). After 20mins the reaction was allowed to warm to room temperature prior to dilution with EtOAc (100ml). The crude reaction mixture was washed with saturated aqueous NaHCO₃ (20ml) and brine (20ml), dried (MgSO₄) filtered and concentrated *in vacuo*. The residual foam was chromatographed (SiO₂; EtOAc) to give the title compound as a white powder (511mg, 0.86mmol, 95%). δ H (CDCl₃, 300K) 8.48 (2H, s), 8.05 (1H, s br), 7.52 (2H, d J 8.4Hz), 7.04 (2H, d J 8.5Hz), 5.81 (1H, d br, J 8.3Hz), 4.98-4.91 (1H, m), 4.21 (2H, q, J 7.1Hz),

30

3.21 (2H, d J 5.3Hz), 1.70-1.66 (4H, m), 1.53-1.44 (4H, m), 1.28 (3H, t J 7.1Hz), 1.20-1.16 (2H, m); m/z (ES⁺, 70V) 597.9 and 595.0 (MH⁺).

INTERMEDIATE 6

5 (2S)-2-(2-Bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoic acid

The compound of intermediate 5 (511mg, 0.86mmol) in THF (5ml) was treated in a single portion with LiOH.H₂O (50mg, 1.19mmol) in H₂O (1ml) and the reaction stirred at room temperature for 2h. The reaction was then
 10 quenched by the addition of HOAc (glacial, 1ml) and the volatiles removed *in vacuo*. Water (10ml) was then added to the residue to effect precipitation. The precipitate was collected by vacuum filtration and the residue washed with water (2 x 5ml). Drying under vacuum gave the title compound as a fine white solid (421mg, 0.74mmol, 87%). δ H (DMSO d_6 ,
 15 390K) 10.34 (1H, s), 8.67 (2H, s), 7.53 (2H, s br), 7.26 (2H, d J 8.26Hz), 4.67 (1H, m), 3.26-3.22 (1H, m), 3.13-3.08 (1H, m), 1.67-1.21 (10H, m); m/z (ES⁺, 70V) 569.9 and 567.9 (MH⁺).

EXAMPLE 1

20 2-Hydroxyethyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

To a solution of the compound of Intermediate 6 (0.5g, 0.89mmol) in DMF (2ml) was added EDC (190mg, 0.97mmol), HOBT (140mg, 1.03mmol) and ethylene glycol (2.5ml). The mixture was stirred at room temperature for
 25 48h then partitioned between EtOAc (15ml) and water (10ml). The aqueous layer was separated and the organics washed with water (3 x 5ml), brine (10ml), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give a crude solid. The crude was chromatographed (SiO₂; EtOAc) to give the title compound as a white powder (287mg, 53%). δ H (300MHz, DMSO
 30 d_6) 8.88 (1H, d, J 9.2Hz), 8.79 (2H, s), 7.59 (1H, d, J 8.5Hz), 7.26 (2H, d, J 8.5Hz), 4.86 (1H, m), 3.62 (1H, m), 3.25 (1H, dd, J 14.0, 4.6Hz), 3.04 (1H, dd, J 14.0, 9.4Hz), 1.58-1.79 (6H, m), 1.37 (1H, d, J 12.7Hz), 1.11 (2H, br); m/z (ES⁺, 70V) 610 (MH⁺).

35 EXAMPLE 2

Methyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

Using a similar procedure to that for the preparation of the ester of Example 1 [acid (0.50g, 0.9mmol), EDC (0.19g), HOBT (0.14g), methanol (1.0ml), DMF (5ml)] was prepared the title compound (0.42g, 80%). ¹H NMR (400MHz, d6 DMSO) δ 1.17 (2H, br), 1.38 (1H, d, J=11.8Hz), 1.58-1.80 (7H, m), 3.05 (1H, dd, J=9.6, 14.8Hz), 3.25 (1H, dd, J=4.6, 14.0Hz), 3.78 (3H, s), 3.62 (1H, m), 4.87 (1H, m), 7.29 (2H, d, J=8.4Hz), 7.62 (2H, d, J=8.4Hz), 8.83 (2H, s), 8.95 (1H, d, J=9.2Hz). *m/z* (ESI, 70V) MH⁺ 580

EXAMPLE 3

Butyl (2S)-2-(2-bromo-3-oxo-spiro[3.5]non-1-en-1-ylamino)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

Using a similar procedure to that for the preparation of the ester of Example 1 [acid (0.50g, 0.9mmol), EDC (0.19g), HOBT (0.14g), n-butanol (1.0ml), DMF (5ml)] was prepared the title compound (0.48g, 86%). ¹H NMR (400MHz, d6 DMSO) δ 0.91 (3H, t, J = 7.4Hz), 1.18 (1H, br), 1.47-1.82 (13H, m), 3.09 (1H, dd, J=9.6, 13.8Hz), 3.25 (1H, dd, J=5.1, 13.8Hz), 4.18 (2H, t, J = 6.3), 4.84 (1H, m), 7.31 (2H, d, J=8.5Hz), 7.62 (2H, d, J=8.5Hz), 8.84 (2H, s), 8.95 (1H, d, J=9.1Hz). *m/z* (ESI, 70V) MH⁺ 622

The following cellular assays can be used to demonstrate the potency and selectivity of the compounds according to the invention. In each of these assays an IC₅₀ value was determined for each test compound and represents the concentration of compound necessary to achieve 50% inhibition of cell adhesion where 100% = adhesion assessed in the absence of the test compound and 0% = absorbance in wells that did not receive cells.

30 $\alpha_4\beta_1$ Integrin-dependent Jurkat cell adhesion to VCAM-Ig

96 well NUNC plates were coated with F(ab)₂ fragment goat anti-human IgG Fc γ -specific antibody [Jackson Immuno Research 109-006-098: 100 μ l at 2 μ g/ml in 0.1M NaHCO₃, pH 8.4], overnight at 4°. The plates were washed (3x) in phosphate-buffered saline (PBS) and then blocked for 1h in PBS/1% BSA at room temperature on a rocking platform. After washing

- (3x in PBS) 9 ng/ml of purified 2d VCAM-Ig diluted in PBS/1% BSA was added and the plates left for 60 minutes at room temperature on a rocking platform. The plates were washed (3x in PBS) and the assay then performed at 37° for 30 min in a total volume of 200 μ l containing 2.5×10^5
- 5 Jurkat cells in the presence or absence of titrated test compounds.

- Each plate was washed (2x) with medium and the adherent cells were fixed with 100 μ l methanol for 10 minutes followed by another wash. 100 μ l 0.25% Rose Bengal (Sigma R4507) in PBS was added for 5 minutes at
- 10 room temperature and the plates washed (3x) in PBS. 100 μ l 50% (v/v) ethanol in PBS was added and the plates left for 60min after which the absorbance (570nm) was measured.

$\alpha_4\beta_7$ Integrin-dependent JY cell adhesion to MAdCAM-Ig

- 15 This assay was performed in the same manner as the $\alpha_4\beta_1$ assay except that MAdCAM-Ig (150ng/ml) was used in place of 2d VCAM-Ig and a sub-line of the β -lympho blastoid cell-line JY was used in place of Jurkat cells. The IC₅₀ value for each test compound was determined as described in the $\alpha_4\beta_1$ integrin assay.

20 $\alpha_5\beta_1$ Integrin-dependent K562 cell adhesion to fibronectin

- 96 well tissue culture plates were coated with human plasma fibronectin (Sigma F0895) at 5 μ g/ml in phosphate-buffered saline (PBS) for 2 hr at 37°C. The plates were washed (3x in PBS) and then blocked for 1h in 100 μ l PBS/1% BSA at room temperature on a rocking platform. The
- 25 blocked plates were washed (3x in PBS) and the assay then performed at 37°C in a total volume of 200 μ l containing 2.5×10^5 K562 cells, phorbol-12-myristate-13-acetate at 10ng/ml, and in the presence or absence of titrated test compounds. Incubation time was 30 minutes. Each plate was fixed and stained as described in the $\alpha_4\beta_1$ assay above.

30 $\alpha_m\beta_2$ -dependent human polymorphonuclear neutrophils adhesion to plastic

- 96 well tissue culture plates were coated with RPMI 1640/10% FCS for 2h at 37°C. 2×10^5 freshly isolated human venous polymorphonuclear neutrophils (PMN) were added to the wells in a total volume of 200 μ l in

the presence of 10ng/ml phorbol-12-myristate-13-acetate, and in the presence or absence of test compounds, and incubated for 20min at 37°C followed by 30min at room temperature. The plates were washed in medium and 100μl 0.1% (w/v) HMB (hexadecyl trimethyl ammonium bromide, Sigma H5882) in 0.05M potassium phosphate buffer, pH 6.0 added to each well. The plates were then left on a rocker at room temperature for 60 min. Endogenous peroxidase activity was then assessed using tetramethyl benzidine (TMB) as follows: PMN lysate samples mixed with 0.22% H₂O₂ (Sigma) and 50μg/ml TMB (Boehringer Mannheim) in 0.1M sodium acetate/citrate buffer, pH 6.0 and absorbance measured at 630nm.

αIIb/β₃ -dependent human platelet aggregation

Human platelet aggregation was assessed using impedance aggregation on the Chronolog Whole Blood Lumiaggregometer. Human platelet-rich plasma (PRP) was obtained by spinning fresh human venous blood anticoagulated with 0.38% (v/v) tri-sodium citrate at 220xg for 10 min and diluted to a cell density of 6 x 10⁸/ml in autologous plasma. Cuvettes contained equal volumes of PRP and filtered Tyrode's buffer (g/liter: NaCl 8.0; MgCl₂.H₂O 0.427; CaCl₂ 0.2; KCl 0.2; D-glucose 1.0; NaHCO₃ 1.0; NaHPO₄.2H₂O 0.065). Aggregation was monitored following addition of 2.5μM ADP (Sigma) in the presence or absence of inhibitors.

In the above assays compounds of the invention such as the compounds of the Examples generally have IC₅₀ values in the α₄β₁ assay of 1 μM and below and in the α₄β₇ assay of 5μM and below.